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DEFICIENCY ON RESPIRATION OF
SUNFLOWER PLANTS

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY

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CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 561

PAUL R. GLENISTER

Introduction

Iron forms a part of the prosthetic groups of several of the respiratory enzymes found in plant cells—peroxidase, catalase, the cytochromes, and perhaps also cytochrome oxidase. These enzymes have been discussed recently (4, 16).

SOMERS, GILBERT, and SHIVE (14), working with soybean, found the respiration of iron-deficient less than that of normal plants, as might be expected.

The present work deals with a series of tests in which the respiratory gradients (respiration rates of leaves at various nodes along the stem) of green and of chlorotic sunflower plants were measured to determine whether a depression of the respiration of the leaves of the iron-deficient plants occurred in all the leaves or whether the depression was limited, like the chlorosis, to the younger leaves.

To supplement this study of respiration, the iron gradients (percentages of iron in leaves at various nodes along the stem) of the green and chlorotic sunflower plants were measured to determine (a) the extent to which the leaves of the two kinds of plants differed in the amounts of iron they contained and (b) the manner in which the iron was distributed among the leaves. With such information, a relationship might be found between iron content, respiration rate, and chlorosis.

Material and methods

During the summer of 1942, sunflower plants (*Helianthus annuus* L.) were grown from seed from a commercial source. The seeds were first removed from the fruit coats and selected for uniformity. They were planted ten per crock in forty 2-gallon crocks which were filled with pure quartz sand. The sand had been cleaned with dilute acid and alkali solutions in the manner described by MINARIK and SHIVE (8) and had been washed thoroughly with distilled water. From the day of planting on, the crocks were supplied with nutrient solution (table 1). Half of them received a complete solution and the remainder received one to which no iron salt had been added. One liter of nutrient solution was supplied to each crock every morning. This was usually supplemented with one or more liters of distilled water given during the afternoon, depending on the heat of the day. Germination was complete in 3 days. The seedlings were thinned to one per crock after 2 weeks, in this way securing as uniform a set of plants as possible. Three such sets were grown, but not all the plants were used, because of attacks by aphids, mildew, and other agents.

The carbon-dioxide output of the

leaves was measured by using the method and apparatus of MULLISON (9). In this method the carbon dioxide produced by the plant material is determined by measuring the changes it causes in the electrical resistance of a definite amount of a solution of standard alkali kept in an absorption tower at a constant temperature. The leaves were inclosed in 500-ml. erlenmeyer flasks with three-holed rubber stoppers and kept there during the tests. One of the holes in the rubber stopper admitted an inlet tube which conducted air from a soda-lime trap to the

TABLE 1

Composition of complete nutrient solution
(iron salt omitted from minus-iron solution)

Components	Concentration
Ca(NO ₃) ₂	0.006 molar
KH ₂ PO ₄	0.0045
MgSO ₄	0.0045
B (in H ₃ BO ₃).....	0.5 p.p.m.
Mn (in MnCl ₂).....	0.25
Cu (in CuCl ₂).....	0.02
Zn (in ZnCl ₂).....	0.05
Fe (in FeC ₆ H ₅ O ₇).....	0.5

flask. The outlet tube passed through the second hole and led to the absorption tower. The ends of the inlet and outlet tubes were so placed in the flask that the air had to flow through the body of the flask (and thus over the leaf surface) in going from one tube to the other. The third hole was slit all the way to the side of the stopper, so that the stopper could be opened like a book and closed with the petiole of a leaf going through the third hole in it. A small amount of chewing gum effectively sealed the space between the petiole and the rubber. The flasks were supported by ring stands. By means of this method the simultaneous measurement of the carbon-dioxide output of ten intact leaves on a single plant could be made.

The leaves were placed in the flasks at

approximately 12:00 noon, and then kept in the dark during the entire subsequent periods. During the first 20 hours, air circulated through the flasks. Then followed a 4-hour period in which carbon-dioxide-free air passed through the flasks and the pipes of the apparatus. Next the absorption towers were put into the system and the carbon dioxide produced by the leaves collected over a 3-hour period. Fresh weights of the leaves were taken immediately after their removal from the apparatus. Dry weights were taken after the leaves had been in an oven kept at 97° C. for 24 hours.

The iron contents of individual leaves were measured by the official colorimetric method (1), using a ferric-alum solution as standard, as described by SNELL and SNELL (13).

Seventeen green and twenty chlorotic plants, possessing twelve to twenty-five leaves each, were tested for respiration gradients. Ten or eleven leaves per plant were tested for carbon-dioxide production; then all the leaves were analyzed for iron.

Results

Symptoms of iron deficiency were noticeable in the plants of the minus-iron treatment after the third week of growth. At this time the leaves of the fifth, sixth, and seventh nodes were pale green or yellow in color, with the principal veins of a darker green. No death of tissue occurred with the chlorosis. As these leaves matured they gradually assumed a greener color. The leaves which appeared at succeeding nodes behaved similarly; they developed as chlorotic leaves and became greener some time after expansion. As a result of this later greening of leaves originally chlorotic, the younger leaves were the only chlorotic ones of the iron-deficient plants.

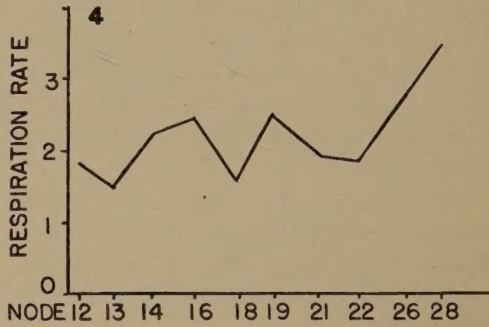
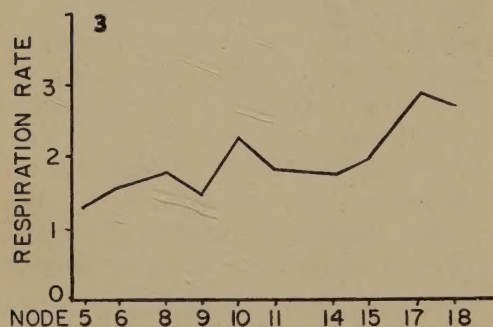
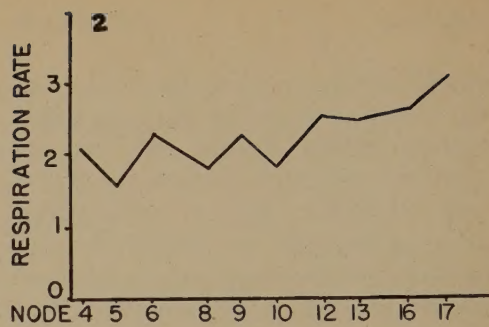
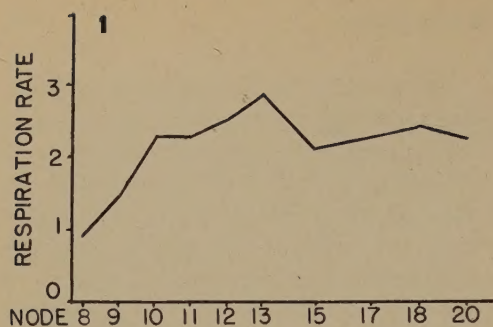
There were no noticeable size differences between the two kinds of plants.

The respiratory gradients of four representative plants of the plus-iron treatment and four of the minus-iron treatment are presented in figures 1-8. The respiration rates (expressed as milligrams of carbon dioxide per gram of dry weight per hour) are plotted along the vertical axes and the numbers of the nodes at which the leaves occurred are plotted along the horizontal axes. The node number was determined by designating the cotyledonary node number 1, and continuing the count from this point to the top of the plant.

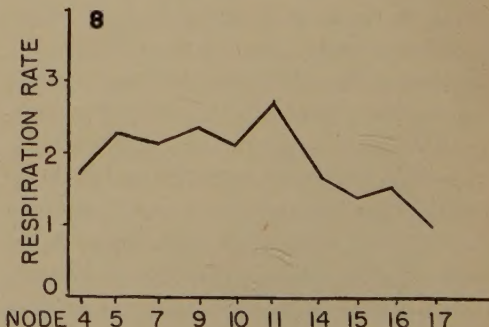
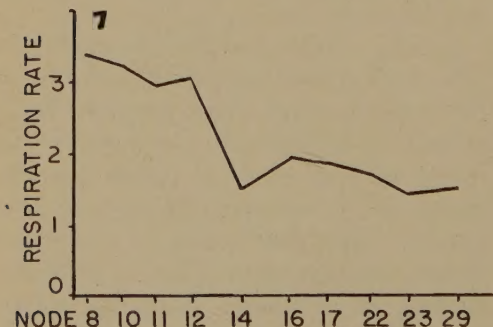
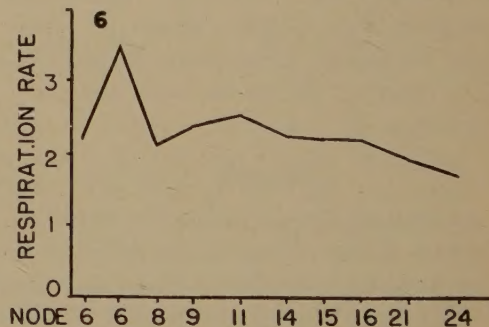
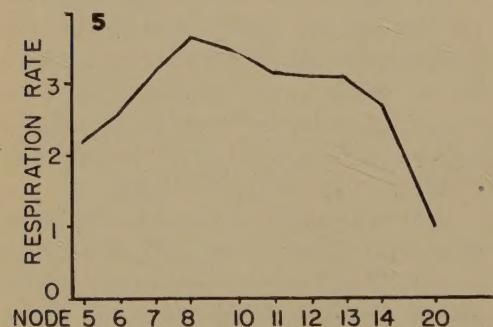
The respiratory gradients for the plants of the plus-iron treatment indicate that the younger leaves were respiring more than the older ones. Such differences are apparently normal, having been reported for other plants (2, 3, 6, 10, 11, 12). The respiratory gradients for the iron-deficient plants indicate that the younger and chlorotic leaves were respiring as much as or less than the older leaves. These gradients are abnormal and indicate a depression of the respiration of the younger chlorotic leaves.

Leaves at adjacent nodes on the same plant may differ greatly in respiration rate. This causes the respiratory gradients to be irregular but does not affect the general trend of the gradients of the two kinds of plants.

Figure 9 is based on the iron gradients of a plant of each treatment, both plants being the same age. These gradients illustrate four findings: (a) the lower older leaves of both kinds of plants contain about ten times as much iron as the younger upper leaves; (b) the gradients of iron content from older to younger leaves are in gradually decreasing order; (c) the leaves of the plant grown with complete nutrient solution contain (with



FIGS. 1-4.—Plus-iron plants: Fig. 1, plant 15, set 1. Fig. 2, plant 6, set 2. Fig. 3, plant 8, set 2. Fig. 4, plant 12, set 2.



FIGS. 5-8.—Iron-deficient plants: Fig. 5, plant 13, set 1. Fig. 6, plant 16, set 1. Fig. 7, plant 18, set 1. Fig. 8, plant 5, set 2.

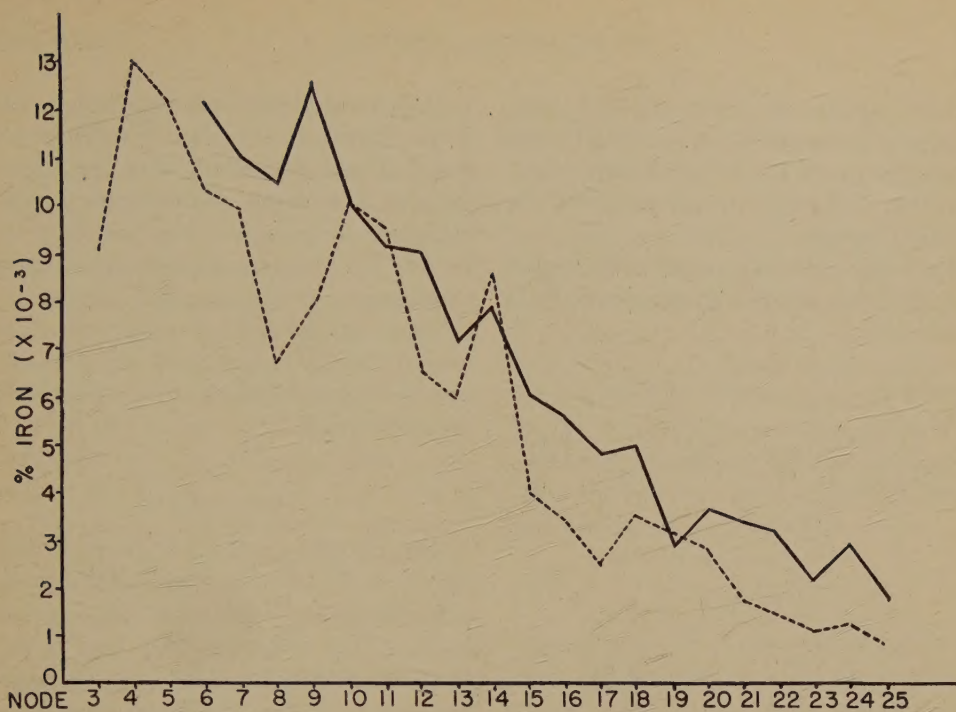


FIG. 9.—Iron gradients in two plants of equal age. Solid line, plus-iron plant; broken line, iron-deficient plant.

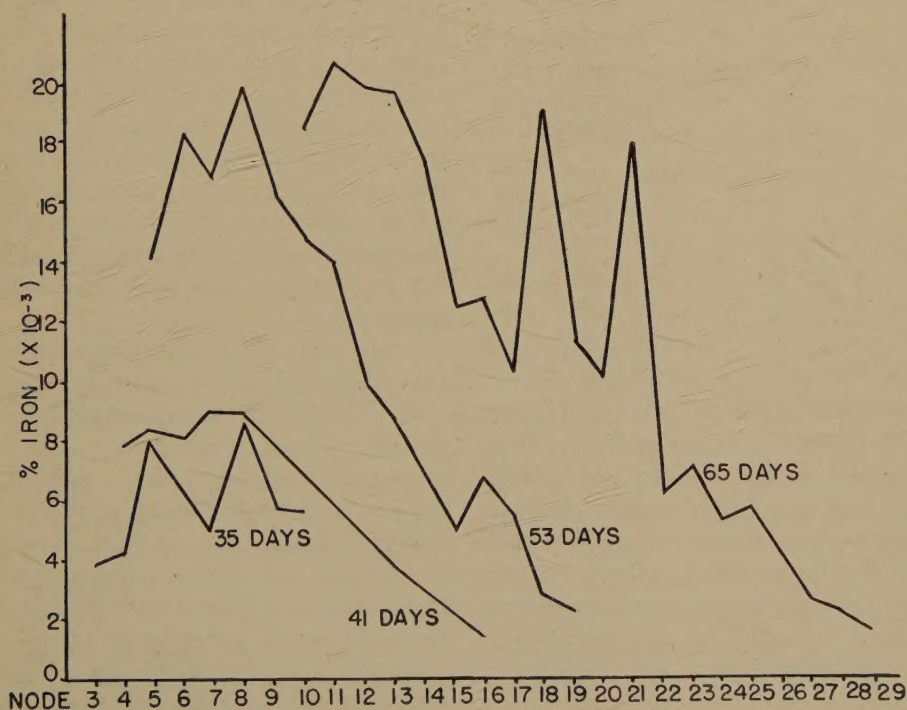


FIG. 10.—Total iron content gradients of plus-iron plants of different ages

a few exceptions) from slightly more than once to more than twice as much iron as the leaves of the iron-deficient plant; (d) this difference is greatest in the younger leaves.

Both the chlorosis and the depression of respiration of the iron-deficient plants

TABLE 2

Node	Green weight (gm.)	H ₂ O (%)	Dry matter (%)	Fe ($\times 10^{-3}$) (%)	Respiration rate
Typical plus-iron plant					
5.....	1.346	84.62	15.38	12.04	1.3
6.....	1.819	86.36	13.64	16.98	1.6
8.....	3.219	87.60	12.40	14.95	1.8
9.....	3.940	87.60	12.40	9.79	1.4
10.....	4.583	86.82	13.18	10.28	2.2
11.....	4.157	87.01	12.99	8.93	1.8
14.....	3.214	85.96	14.04	7.52	1.7
15.....	3.454	85.72	14.28	5.94	1.9
18.....	2.016	85.06	14.94	8.71	2.9
19.....	1.673	84.93	15.07	6.86	2.9
Typical iron-deficient plant					
4.....	1.629	85.87	14.13	11.28	1.8
5.....	1.749	87.53	12.47	11.93	2.3
7.....	4.202	88.83	11.17	8.07	2.2
9.....	4.662	89.44	10.56	10.12	2.6
10.....	4.744	86.81	13.19	8.10	2.4
11.....	4.781	88.72	11.28	7.75	2.8
14.....	5.776	88.22	11.78	4.65	1.7
15.....	4.446	87.69	12.31	3.78	1.4
16.....	3.296	88.53	11.47	2.76	1.4
17.....	2.834	88.14	11.86	3.16	1.0

occur in the leaves with the greatest deficiency in iron—the younger leaves. Figure 10 presents the iron gradients of four plants of similar treatment but of different age. It shows that the iron content of leaves at corresponding nodes is proportional to the age of the plants compared. The older a plant is, the more iron is contained in its leaves.

Table 2 gives the data for green weight, water content, dry-matter con-

tent, iron content, and respiration rate for a representative plant of each treatment. These data show that the differences in iron supply did not have a great influence on growth of the plants as reflected by these measurements. Direct comparisons of this sort are impossible, because corresponding nodes were not used in every plant and because the plants were of different ages when tested for respiration.

Discussion

The effects resulting from iron deficiency, both chlorosis effects and respiration effects, are localized in the younger leaves. This indicates that iron is not transferred from the older to the younger parts, and that a continuous supply of iron must be available if the younger leaves are to have normal color and normal respiration rate.

The gradients of iron content shown in figure 9 support this interpretation. The gradient for the iron-deficient plant follows the same pattern as that of the plant grown with complete nutrient. If relocation of iron from older to younger leaves had occurred in the iron-deficient plant, its gradient would have been quite different, especially in the region of the older leaves.

It appears to be a general phenomenon that the deficiency symptoms of non-reutilized elements occur primarily in the younger leaves. Chief among such elements are calcium, boron, manganese, copper, and iron. Elements which can undergo reutilization, such as nitrogen, phosphorus, potassium, and magnesium, have deficiency symptoms which occur primarily on the older leaves (5, 7).

In the present work the chlorotic leaves of the iron-deficient plants were found to contain some iron. Lack of it

must have been relative rather than absolute. The problem is therefore one of suggesting a reason for the inactivity of the iron in these leaves. Two mechanisms of inactivation of iron have been suggested by previous investigators.

The first is that the iron is precipitated in the conducting elements because of the high pH of the plant sap. Such precipitated iron would presumably be of no value to the plant. Chlorosis of this type is usually caused by a high calcium content of the soil (2).

The second mechanism of iron inactivation is that involving the iron-manganese ratio. It has been shown by solution culture methods that an iron-deficiency chlorosis will occur if iron is not maintained in the plant in the ratio of 2:1 with manganese (14). The postulated mechanism (15) is briefly as follows. Active iron is ferrous iron, and it tends to be kept in that state by the reducing mechanisms of the tissues. Manganese, because it has a greater oxidation potential than iron, will—when present in excess in relation to iron—tend to keep the iron in the inactive ferric state.

In the present study the first mechanism of inactivation can hardly apply. The concentration of calcium in the nutrient solution was not excessive, and the frequent applications of distilled water which were applied would have kept the calcium from accumulating to an excessive concentration.

The second mechanism of inactivation seems to be the more likely one in the present case. The ratio of iron to manganese in the nutrient solution applied to the iron-deficient plants was far below 2:1. A difficulty with this second mechanism, however, is its premise that active iron is in the ferrous state. While such an assumption may apply to the function of iron in chlorophyll synthesis, it can

hardly apply to its function in respiration. The iron in the cytochrome system functions by way of a cyclic change from the ferric to the ferrous condition. It is the same in the enzyme catalase. Peroxidase occurs and functions only in the ferric condition. It seems more likely that the observed depression of respiration may have been caused by the action of the manganese in preventing or hindering the cyclic valence changes of the iron-containing enzymes in which such changes occur, rather than by the removal of iron from an active ferrous state.

Summary

1. Sunflower plants were grown in sand culture with nutrient solution. One group had iron salt as ferric citrate added to the nutrient solution; the other had no iron salt added.

2. The plants of the minus-iron treatment developed the typical chlorosis symptoms of iron deficiency; the controls were of normal color.

3. Respiration rate was depressed in the young chlorotic leaves of the iron-deficient plants.

4. The chlorotic leaves of the iron-deficient plants contained only half as much iron as comparable leaves of control plants. No relocation of iron from older to younger leaves occurred in the iron-deficient plants. In both the gradients were of decreasing iron content in going from the bottom to the top of the plants.

5. The iron contents of the leaves of a given plant of either treatment were found to be related to the ages of the leaves. The older contained more iron than the younger leaves.

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